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Molecular cloning, characterization, and chromosomal mapping of a novel human gene (GTF3A) that is highly homologous to Xenopus transcription factor IIIA.

Arakawa H, Nagase H, Hayashi N, Ogawa M, Nagata M, Fujiwara T, Takahashi E, Shin S, Nakamura Y.

Department of Biochemistry, Cancer Institute, Tokyo, Japan.

We have isolated a novel human cDNA that is highly related to Xenopus transcription factor IIIA (TFIIIA). This clone contains an open reading frame of 1,269 nucleotides encoding 423 amino acids, including nine repeats of the Cys2His2-type of zinc-finger domain. A comparison of its sequence with Xenopus TFIIIA revealed 63% identity in nucleic acids and 58% identity in amino acids over a large portion of the gene and predicted peptide, indicating that the human homologue is likely to function as a transcription factor. The zinc-finger domains of the predicted protein also showed homology with those of human genes such as WT1, transcriptional repressor YY1, and MYC-associated zinc-finger protein (MAZ). Northern analysis showed expression in various tissues examined. The human TFIIIA gene (GTF3A) was localized to chromosome band 13q12.3-->q13.1 by fluorescent in situ hybridization (FISH).

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EUROPEAN JOURNAL OF BIOCHEMISTRY, (1990 Nov 26) 194 (1) 167-76.
NUCLEIC ACIDS RESEARCH, (1995 Jan 11) 23 (1) 109-16.

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Nucleic Acids Research, 1995, Vol. 23, No. 1 109–116

Human TFIIIA alone is sufficient to prevent nucleosomal repression of a homologous 5S gene

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Received September 22, 1994; Revised and Accepted November 24, 1994

ABSTRACT

Plasmid DNA harbouring the human 5S rRNA gene was assembled into nucleosomes using either *Xenopus* S150 extracts or purified core histones in the presence of pectin. In both cases reconstitution of nucleosomes led to a complete repression of transcription. This repression could be efficiently counteracted by pre-incubating the template DNA with highly purified hTFIIIA which allowed the protein to bind to the ICR of the 5S gene. By using an efficient and well-defined *in vitro* reconstitution system based on isolated core histones in the presence of pectin, which is devoid of endogenous transcription factors, we demonstrate here for the first time that human TFIIIA alone is sufficient to prevent nucleosomal repression of h5S gene transcription and that additional pol III transcription factors are not required to achieve this effect. Additionally, we investigated the binding of hTFIIIA to a mononucleosome reconstituted on the human 5S gene. DNAse I footprinting experiments reveal that the entire ICR of the human 5S gene is covered by the nucleosome, thereby precluding the subsequent binding of human TFIIIA to the promoter of the 5S gene.

INTRODUCTION

The chromatin organization of genes is of great importance with respect to regulatory processes like replication and transcription (1) and several studies have presented evidence that the initiation of transcription is strongly affected by the formation of nucleosomes (2–7). Since transcription of eukaryotic genes *in vivo* occurs in a nucleosomal environment, the question arises how *trans*-acting factors gain access to regulatory DNA sequences embedded in densely packed chromatin and whether individual transcription factors or the complete initiation complex must bind in order to displace nucleosomes or alter chromatin structure.

Previous studies have shown that formation of transcription complexes and nucleosome assembly can compete for one another in the wake of the replication fork (8,9) and that pre-assembled transcription complexes are destroyed during replication (10–12). In addition, mechanisms must have evolved which render existing chromatin accessible for transcription in the absence of replication. Recently it has been shown by Chen *et al.* (13) that nucleoplasmin from *Xenopus laevis* oocytes is capable of removing histone H2A–H2B dimers from the histone octamer, subsequently allowing the formation of triple complexes between transcription factors, the remaining histone tetramer and the DNA. Another possibility involves the acetylation of histones (14–18) or other forms of modification (19). It is conceivable that a broad spectrum of such mechanisms exist, which may vary from gene to gene. A suitable system which has previously been employed to investigate these questions concerns the regulation of expression of the 5S rRNA genes (20). Although considerable information has been accrued concerning the interaction of transcription factors IIIA, IIIB and IIIC with the 5S gene promoter (21,22) and their role during the assembly of chromatin on the 5S gene from *X.laevis* (23,24), the results obtained are discussed quite controversially. Initially, Gottesfeld and Bloomer (25) found that pre-incubation of *X.laevis* oocyte TFIIIA with a 5S gene prior to nucleosome assembly, counteracted the inhibitory nucleosomal influence on transcription. In contrast, Tremethick *et al.* (26) reported that transcription factor IIIA alone does not prevent nucleosomal repression of transcription of the *X.laevis* somatic 5S rRNA gene and that the entire transcription complex is required to achieve anti-repression. In agreement with these findings, Felts *et al.* (27) showed that pre-binding of yeast TFIIIA is not sufficient to maintain an active transcriptional state after chromatin assembly.

Among other possibilities, the apparent discrepancies in the literature could possibly be related to species differences observed for TFIIIA. This protein was first characterized (28,29) and cloned (30,31) from *X.laevis* oocytes. However, its functional counterpart from human cells seems to differ in size from the amphibian oocyte protein, although different molecular masses of 35 (32) and 42 kDa (33) have also been reported for the human protein. Moreover, TFIIIA from *Saccharomyces cerevisiae* has a different molecular weight and completely different amino acid sequence from that of the amphibian oocyte protein (34,35) although the resulting structure, based on nine zinc-finger motifs, appears to be very similar.

The ability of human TFIIIA to act as an anti-repressor of nucleosomal inhibition of 5S gene transcription has hitherto not been investigated. We employed a plasmid containing a human genomic 5S rRNA gene and analysed which factors are required to prevent nucleosomal repression of 5S rRNA synthesis *in vitro*.

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We report here for the first time that human TFIIIA is a strong anti-repressor and that binding of the protein alone is sufficient to prevent nucleosomal repression of transcription.

In addition we investigated the ability of hTFIIIA to recognize its cognate binding sequence in the context of a nucleosome positioned on the human 5S gene. It was previously shown that 5S genes strongly position nucleosomes (36) and that TFIIIA from *Xenopus* oocytes differs in its ability to bind to a 5S nucleosome depending on the exact positioning of the histone octamer (37–39). Our results show that the nucleosome occupies the human 5S promoter in a way precluding the subsequent binding of human TFIIIA.

MATERIALS AND METHODS

Plasmid DNA and DNA fragments

The 5S rRNA gene derived from human placenta DNA was cloned into the *Bam*HI–*Sac*I site of a Bluescript vector (Stratagene) as described previously (40). For the electrophoretic mobility shift experiments and for the footprints a 270 bp *Ava*II–*Hin*fI restriction fragment containing the h5S gene including the whole ICR was prepared, terminally labelled with [γ-^{32}P]ATP and re-cut with *Sma*I to obtain a 244 bp fragment labelled at one side. Finally, the fragment was purified on a 5% polyacrylamide gel. For the transcription experiment outlined in Figure 5B a *Sal*I–*Alu*I fragment of the VAI RNA gene subcloned into the *Hin*cII site of pUC 18, was used.

Reconstitution of chromatin

Two different methods were used to reconstitute nucleosomes on plasmid DNA. Using *X.laevis* extracts (S150) plasmids harbouring the human 5S gene were assembled into chromatin as described previously (41,42). The dose–response curve for efficient inhibition of 5S transcription differed slightly in individual experiments, but on average 5 µl of oocyte extract corresponding to an average protein content of 20 µg led to complete repression of transcription even when the plasmids were not fully assembled into nucleosomes. Reconstitution was started by adding 40 mM creatine phosphate, 3 mM ATP, 1.6 ng creatine kinase and 3 mM $MgCl_2$ to the reaction mixtures. The transcription buffer (see below) was complemented with 1 mM EDTA and 10 mM β-glycerophosphate (Sigma) in a total volume of 65 µl. Samples were incubated for 4 h at 30°C in order to allow the nucleosomes to assemble on the template DNA. Alternatively, reconstitution was achieved according to the method described by Stein (43) with a few modifications. Histones from HeLa cell nuclei were bound to hydroxyapatite. The linker histones were removed by washing with 0.7 M NaCl buffer. The core histones were eluted at 2.5 M NaCl and were concentrated using Filtron macrosep concentrators by centrifugation at 5000 r.p.m. for about 4 h in a Sorvall HB 4 rotor. Quality and purity of the isolated core histones were checked by SDS–PAGE. For an efficient reconstitution, plasmid DNA and histones were mixed at a molecular mass ratio of 1:2, in the presence of relatively high amounts of pectin (40-fold in comparison to DNA concentration) and 250 mM NaCl. Pectin has been shown to serve as a histone sink preventing aggregation of histones at low ionic strength (44). The mixture was then dialyzed against transcription buffer for at least 3 h.

Reconstitution of mononucleosomes on labelled DNA fragments

Nucleosomal cores from H1/H5-stripped donor chromatin from avian erythrocytes were prepared as described (45,46) with a few modifications. Nuclei from duck erythrocytes (160 OD_{260} U) were digested with 250 U micrococcal nuclease (Sigma) at 37°C for 5 min. The reaction was stopped by addition of 0.5 M EDTA, pH 8.0. After centrifugation at 8000 *g*, the resulting pellet was suspended in 0.2 mM EDTA and the chromatin was obtained by intensive pipetting for several times. After a second centrifugation at 8000 *g* the chromatin remained in the supernatant which was subsequently loaded onto a Sephacryl S-400 gel filtration column. The S-400 column was equilibrated with 5 mM Tris–HCl, pH 7.5, 2.5 mM EDTA, 550 mM NaCl, 0.2 mM PMSF and 0.2 mM β-mercaptoethanol. The quality of the prepared chromatin was checked by 17.5% SDS–PAGE. Those chromatin fractions which were found to contain only core histones were pooled and concentrated using Centricon microconcentrators (Amicon). For chromatin reconstitution on 5S DNA fragments we combined 200–300 ng of the terminally labelled h5S fragment (containing at least 2×10^6 c.p.m.) with 40 µg of the concentrated donor chromatin in reconstitution buffer (13 mM Hepes, pH 7.5, 47 mM Tris–HCl, pH 7.5, 2 M NaCl, 1 mM EDTA and 20 µM β-mercaptoethanol). Reconstitution of nucleosomes was achieved by a salt gradient dialysis from 2 M to 250 mM NaCl over at least 10 h. The reconstituted material was separated from the remaining free DNA and high molecular aggregates by centrifugation through a 5–30% glycerol gradient for 15 h at 55 000 r.p.m. in a Beckman SW 60 rotor at 4°C.

Purification of hTFIIIA and generation of antibodies

Human TFIIIA was purified from HeLa cytoplasmic extracts (S100) by phosphocellulose chromatography as described previously (32). Activity of hTFIIIA was eluted with 0.1 M KCl (PC A). Human TFIIIB and hTFIIIC activities were eluted with increasing salt concentrations (0.35 and 0.6 M KCl). For further purification, the PC A fraction was re-chromatographed on phosphocellulose with 0.6 M KCl and hTFIIIA was finally eluted with 1 M KCl (PC AD). Polyclonal antibodies against proteins in this fraction were raised in rabbits as described previously (47).

In vitro transcription

After pre-incubation of template DNA with protein fractions and subsequent nucleosome assembly as illustrated in Figure 3A, the transcription reactions were performed essentially as described previously (48). The transcription buffer used in our assays contained 20 mM Tris, pH 7.9, 60 mM KCl, 5 mM $MgCl_2$, 10% glycerol, 0.2 mM PMSF and 3 mM dithiothreitol.

Micrococcal nuclease cleavage and analyses of DNA topology

Chromatin reconstitution by *X.laevis* oocyte extract was obtained under the conditions described above prior to nuclease digestion. After digestion, RNAse treatment and proteinase K digestion was conducted overnight at 37°C. After DNA extraction the samples were loaded onto a 1.2% agarose gel and electrophoresed in 0.5× TBE buffer at 50 V overnight. DNA topology was investigated by reconstitution of the plasmids with increasing amounts of oocyte extract as indicated in Figure 1. Samples were digested

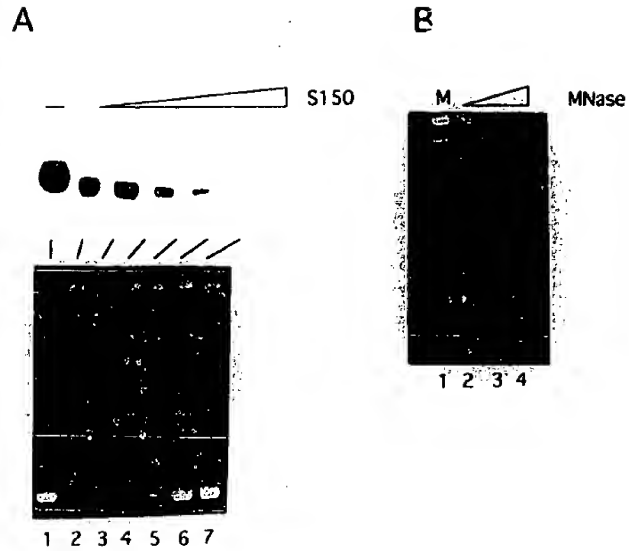

Figure 1. Oocyte S150 extract inhibits transcription of a human 5S gene. (**A**) Decrease of 5S gene transcription (upper panel). The topological change in the plasmid DNA under transcriptional conditions is shown at the bottom of the figure. The samples contained 300 ng plasmid DNA harbouring the human 5S gene and 0 (lane 1), 2 (lane 2), 5 (lane 3), 10 (lane 4), 15 (lane 5), 30 (lane 6) and 50 (lane 7) µl of oocyte S150 extract. (**B**) The oocyte extract assembles the h5S plasmids into nucleosomes. The figure shows the micrococcal nuclease cleavage pattern of the reconstituted samples under the same conditions shown in (A). Lane 1, marker DNA (multiples of 100 bp); lanes 2–4, 300 ng plasmid DNA was cleaved with 1, 2.5 and 5 U of micrococcal nuclease for 1 min at room temperature. The cleavage reaction was stopped with a 75 mM EDTA–900 mM ammonium acetate buffer, pH 8 (see Materials and Methods).

overnight with proteinase K and the DNA was processed as described, with the exception that the gel was run for about 20 h at 50 V. All gels were stained with ethidium bromide. To check the linking number change as a consequence of nucleosome formation, a two-dimensional gel electrophoresis was performed including 4 µM chloroquin in the second dimension as described (49).

Restriction analyses with *ScaI*

The availability of a *ScaI* cleavage site was proven by digestion of the reconstituted samples (300 ng plasmid DNA and varying amounts of oocyte extract) with 0.3 U *ScaI* (obtained from Amersham) for 1.5 h at 37°C in transcription buffer.

Electrophoretic mobility shift analyses (EMSA)

Binding of hTFIIIA to the gel-purified *HinfI–SmaI* 244 bp fragment containing the human genomic 5S gene was assayed either as such or to the same DNA fragment which was organized into a nucleosome. For this purpose, the protein fraction containing hTFIIIA (PC AD) was pre-incubated with 0.3 µg pUC9 DNA, 20 µg BSA and 1 mM DTT at 30°C for 10 min. Subsequently the labelled DNA fragment (free or nucleosomally organized) was added and the incubation was prolonged at 30°C for 45 min. Following incubation, the samples were loaded onto a 4% (w/v) native polyacrylamide gel. The gel was pre-run for 30 min and 100 V at 4°C with a buffer containing 7 mM Tris–HCl, pH 7.9, 1 mM EDTA, 3 mM sodium acetate. After loading the samples, the gel was run at 150 V at 4°C for 2–3 h. The gel was subsequently transferred to Whatman paper, dried and autoradiographed overnight at –80°C with an intensifying screen.

DNAse I footprint analyses

The end-labelled 244 bp fragment of the h5S gene described above was incubated with 70 µl phosphocellulose AD fraction containing hTFIIIA under the conditions described for the EMSA. This sample and the free 5S DNA were cleaved with 10 ng of DNAse I dissolved in 25 mM $MgCl_2$ and 0.5 mM $CaCl_2$. In the case of the octamer, digestion was carried out with up to 70 ng of DNAse I. Cleavage was conducted for 1 min at room temperature and the reaction was stopped with 100 µl of stop solution [450 mM sodium acetate, 0.1% SDS (w/v), 10 mM EDTA]. The samples were incubated with proteinase K for 2 h and then extracted with phenol/chloroform/isoamyl alcohol (50:50:1). Finally the samples were precipitated with ethanol/sodium acetate (30:1) and the pellets were dissolved in 5 µl of a 95% formamide loading buffer. Prior to loading onto a 6% (w/v) sequencing gel the samples were denatured at 95°C. The gels were autoradiographed with intensifying screens for 2 days (free 5S DNA sample and the hTFIIIA footprint reaction) or several days in the case of the nucleosomal footprint.

RESULTS

Repression of h5S rRNA gene transcription by increasing nucleosomal densities after *in vitro* reconstitution of chromatin

Two different systems were used to reconstitute nucleosomes on the h5S gene *in vitro*. On the one hand we used crude cytoplasmic extracts (S150) derived from stadium 5–6 *X.laevis* oocytes to assemble the 5S gene into nucleosomes (42). On the other hand we combined isolated HeLa core histones with the acidic macromolecule pectin at a NaCl concentration of 250 mM to assemble nucleosomes on plasmid DNA. Subsequent dialysis against transcription buffer led to the formation of defined nucleosomal structures. The latter method has the advantage of a biochemically well-defined system, but leads to closely spaced nucleosomes, whereas the chromatin assembled by oocyte extract showed nearly physiological spacing.

As shown in Figure 1A, incubation with increasing amounts of oocyte S150 extract led to a complete repression of h5S gene transcription. The decrease in transcription was not due to a hypothetical inhibitor contained in the extract, because transcription of a maxi 5S gene added at the beginning of the transcription reaction remained unaffected (data not shown). The topological change of the template DNA during incubation with S150 under transcriptional conditions is shown at the bottom of the figure and it is obvious that superhelical stress is introduced into the plasmid DNA. The formation of nucleosomes was additionally shown by micrococcal nuclease digestion, which generated the typical pattern of DNA cleavage at a periodicity of 200 bp typical for nucleosomal spacing (see Fig. 1B). The change in linking number towards negative values was proven by two-dimensional gel electrophoresis in the presence of 4 µM chloroquin, which revealed a nucleosomal density of 12–13 nucleosomes/plasmid (data not shown). Basically similar results as shown in Figure 1A were obtained by using isolated core histones to assemble

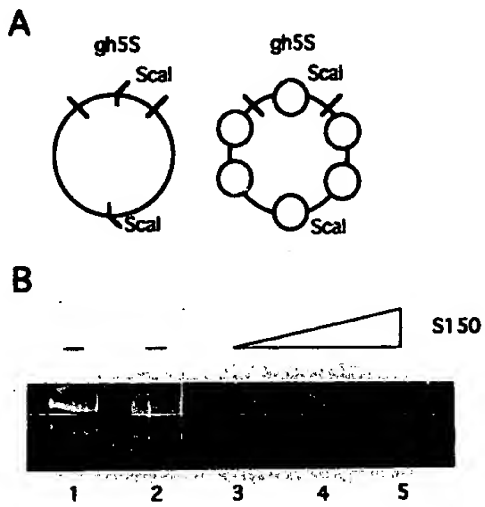

Figure 2. A nucleosome reconstituted over the ICR of the human 5S gene alters the accessibility. (**A**) Schematic diagram of the h5S plasmid with a *ScaI* site within the gene and another in the vector DNA. The small circles in the right part of the figure imply packaging into nucleosomes. (**B**) Increasing amounts of oocyte extract diminish the availability of the restriction sites. The oocyte extract alone (10 µl) added at the beginning of the restriction reaction does not influence enzyme activity (lane 1). The same was proven for the reconstitution buffer alone (lane 2). The h5S plasmids were reconstituted into chromatin and then digested with 0.3 U *ScaI*. Lanes 3–5 correspond to 2, 5 and 10 µl oocyte S150 extract used in the reconstitution assay.

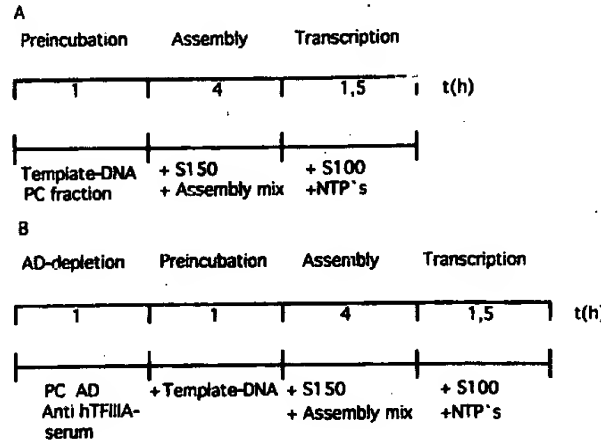

Figure 3. Experimental design for the transcription experiments. (**A**) Standard protocol pursued for the pre-incubation with different phosphocellulose fractions, followed by chromatin assembly and the transcription reaction. Note that the figure describes the conditions for chromatin assembly in the oocyte extract. Alternatively, reconstitution was obtained with isolated core histones in the presence of pectin and salt followed by dialysis for 3 h. (**B**) Outline of the experimental approach for the immunodepletion of hTFIIIA from phosphocellulose fraction AD before starting the pre-incubation step (see text for details).

nucleosomes, in which case we observed little spacing, indicating the crowded package of nucleosomes.

Restriction analysis of the h5S plasmid bearing a *ScaI* cleavage site within the ICR

To further investigate the chromatin organization of the h5S gene, a different experimental approach was taken. Cleavage with *ScaI* at a site within the ICR of the h5S gene and at a corresponding site in the vector generates two fragments when using chromatin-free DNA (Fig. 2A). Assuming that the availability of the cleavage site would be reduced when organized in a nucleosome, the restriction will not yield these two distinct fragments. Figure 2B demonstrates that the restriction efficiency declines after the h5S plasmid has been incorporated into nucleosomes (compare lanes 1 and 2 with 3–5). Neither a conceivable non-specific inhibitor in the S150 extract (lane 1) nor the assembly buffer alone (lane 2) was responsible for the reduction in cleavage activity. It is therefore assumed that the formation of a nucleosome within the ICR of the 5S gene is responsible for the loss of restriction efficiency.

Pre-incubation of a human 5S rRNA gene with a protein fraction highly enriched in hTFIIIA prior to nucleosome assembly fully maintains transcriptional activity

The protein fraction (PC AD) used in these experiments was highly enriched in hTFIIIA activity and was entirely free of cross-contamination by TFIIIB and TFIIIC, as evidenced by reciprocal reconstitution of these fractions and assay for 5S transcription. The standard protocol followed in the pre-incubation experiments is outlined in Figure 3A. Pre-incubation of the human 5S gene with the protein fractions was followed by addition of the isolated core histones from HeLa cell nuclei or oocyte S150 extract, respectively. The transcription reaction was started by the addition of nucleoside triphosphates (including [α-^{32}P]GTP) and HeLa S100 cytoplasmic extract. As shown in Figure 4A, pre-binding of human TFIIIA (PC AD) is sufficient to maintain transcriptionally active 5S templates even after nucleosomal reconstitution in the presence of pectin and 250 mM NaCl, whereas hTFIIIB (PC B) and hTFIIIC (PC C) do not show this anti-repression (compare lanes 3–5 with 7–9 and 11–13, respectively). It should be pointed out in this context that the binding of hTFIIIA to the human 5S promoter is stable under these conditions of ionic strength (32). Chromatin assembly using the oocyte S150 extract led to the same result (Fig. 4B). It is conceivable that the rescue of transcriptional activity could be due to stimulatory effects of the PC AD fraction on the transcriptional machinery present in the S100 extract. We ruled out this possibility by showing that addition of the same amount of PC AD after chromatin assembly revealed no stimulatory activity (see Fig. 4A lane 6 and Fig. 4B lane 7). It should be noted that none of the PC fractions were able to generate h5S transcripts in the oocyte extract without addition of HeLa S100 cytoplasmic extract, indicating that essential components of the transcriptional machinery are either missing or inactive in the oocyte extract (data not shown).

The rescue of transcriptional activity depends on hTFIIIA

To investigate the specificity of the observed anti-repressive effect, we generated polyclonal antibodies against purified hTFIIIA in rabbits. As shown previously, these antisera inhibit binding of human TFIIIA to the 5S gene and its subsequent transcription (47). We used these antibodies to deplete human

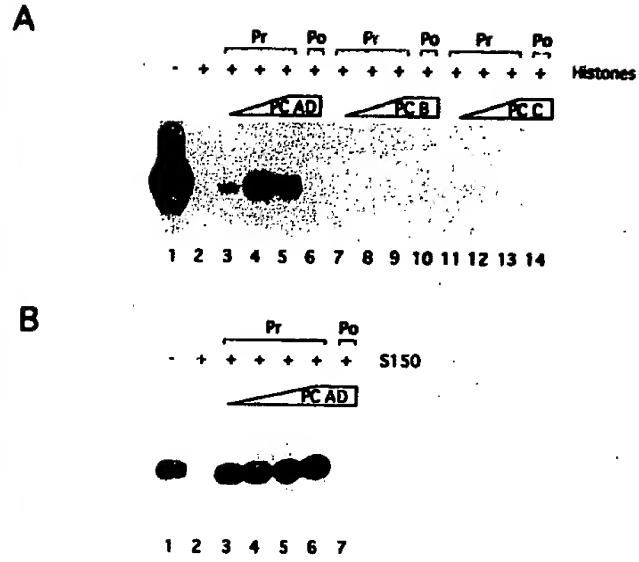

Figure 4. Restoration of transcriptional activity by binding of hTFIIIA prior to nucleosome assembly. (**A**) 300 ng h5S plasmid DNA was pre-incubated with 20, 40 or 50 µl of phosphocellulose (PC) fractions prior to nucleosome assembly with isolated core histones in the presence of pectin and salt. Lanes 3–5, PC AD; lanes 7–9, PC B; lanes 11–13, PC C. The transcription of 300 ng plasmid DNA without pre-incubation and chromatin assembly is shown in lane 1. The inhibition of transcription by histones without pre-incubation with PC fractions is outlined in lane 2. Additional controls were conducted by adding 50 µl of the phosphocellulose fractions as appropriate (as indicated) after chromatin assembly had occurred (lanes 6, 10 and 14). The abbreviations pr (pre) and po (post) indicate the addition of phosphocellulose fractions prior to or after nucleosome assembly. (**B**) This part depicts the same conditions as in (A) with the exception that oocyte S150 extract was used for the chromatin assembly. Lane 1, transcription of the plasmid without pre-incubation and chromatin assembly; lane 2, inhibition of transcription by 5 µl oocyte S150 extract; lanes 3–6, 2, 5, 12 or 20 µl PC AD fraction were contained in the pre-incubation step prior to chromatin assembly with 5 µl oocyte extract; lane 7, 20 µl PC AD fraction were added as a control after chromatin assembly was conducted with 5 µl oocyte extract.

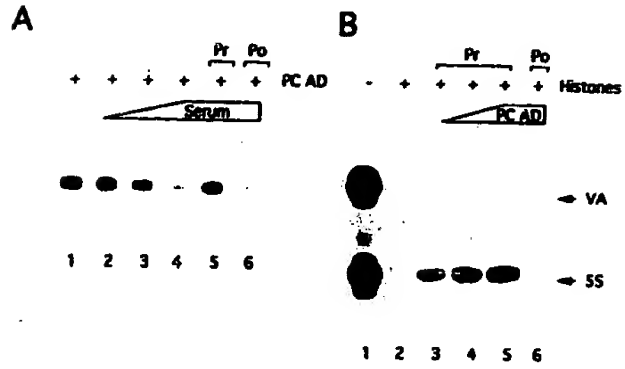

Figure 5. Rescue of transcriptional activity is specific for hTFIIIA. (**A**) Immunodepletion of hTFIIIA from phosphocellulose fraction AD before binding to the 5S template. Before pre-incubating the plasmid DNA with 5 µl PC AD fraction, antiserum directed against the PC AD fraction was added to deplete hTFIIIA from this fraction (lanes 2–4 corresponding to 1, 2 and 6 µl serum). As a control, 300 ng h5S plasmid DNA was pre-incubated with 5 µl undepleted PC AD fraction prior to chromatin assembly with 5 µl oocyte extract (lane 1). Lane 5, after depletion of 5 µl PC AD fraction with 6 µl serum this assay was reconstituted with 5 µl PC AD fraction before pre-incubation (Pr). Lane 6, depletion of 5 µl PC AD fraction with 6 µl serum before pre-incubation. After chromatin assembly with 5 µl oocyte extract, 5 µl PC AD fraction were added (Po). (**B**) Phosphocellulose fraction AD fails to restorate VAI RNA gene transcription. 250 ng h5S and VAI plasmid DNA were transcribed together without pre-incubation with PC AD and in the absence of chromatin assembly (lane 1). Lane 2, transcription of both templates was inhibited by addition of 600 ng isolated histones in the presence of pectin and salt; lanes 3–5, 20, 40 or 50 µl of phosphocellulose fraction AD were present in the pre-incubation step before addition of the histones (Pr); lane 6, 50 µl of fraction AD were added to the transcriptional reaction after chromatin assembly (Po).

TFIIIA from the PC AD fraction prior to pre-incubation with the template DNA (see Fig. 3B). As demonstrated in Figure 5A, addition of these antibodies leads to a progressive loss of transcription, presumably by depleting hTFIIIA and hence allowing the formation of nucleosomes (lanes 2–4). This was proven by restoring transcriptional activity through the addition of purified hTFIIIA prior to pre-incubation with the 5S template (control pr, lane 5). As a second control we added the same amount of undepleted PC AD fraction after nucleosome assembly (control po, lane 6). From the results it is evident that antibodies against hTFIIIA block the function of this protein and hence allow nucleosomal repression of transcription which can be recovered by the addition of the PC AD fraction before starting pre-incubation. Antibodies from pre-immune serum did not exhibit this effect (data not shown).

As an additional and independent way to assess whether the anti-repressive effect of phosphocellulose fraction AD is specific for the 5S gene we simultaneously assayed transcription of a pol III gene which does not require TFIIIA. In this case the VAI gene was used as a control. The results (Fig. 5B) show that under the conditions tested, both genes are transcribed approximately equally well in the same reaction (lane 1). Both genes are completely repressed by the addition of histones (lane 2). While this repression can be counteracted by fraction PC AD in the case of the 5S gene, transcription of the VAI gene remains repressed in the presence of this fraction (lanes 3–5). Addition of fraction AD after the reconstitution reaction (lane 6) does not restore transcription, as was already discussed above. These results show that the anti-repressive effect observed is specific for the 5S gene and that formation of the entire transcription complex on the h5S rRNA gene is not necessary to prevent inhibition of transcription by nucleosomes.

Inability of hTFIIIA to form a stable complex with the 5S gene and the histone octamer

In the following experiment we studied the binding of hTFIIIA to a reconstituted nucleosome formed on a 244 bp fragment of the human 5S gene. As shown in Figure 6, hTFIIIA was found to be unable to bind to nucleosomally packaged 5S DNA (lanes 8–12).

Overlap between the 5S promoter and the nucleosome

To investigate the positioning of the nucleosome in relation to the 5S gene, a footprint assay was performed. As depicted in Figure 7, the reconstituted nucleosome overlaps with the promoter of the human 5S gene, including the entire ICR, as evidenced by the modular appearance of ~10 bp fragments after DNAse I treatment (see horizontal arrows and compare lanes 1 and 2). Lane 3 demonstrates the footprint of human TFIIIA on the coding strand of the h5S gene. In comparison to lane 1, the overlap between the nucleosome and the ICR is clearly visible. This finding strongly supports the data found in the eletrophoretic mobility shift assay

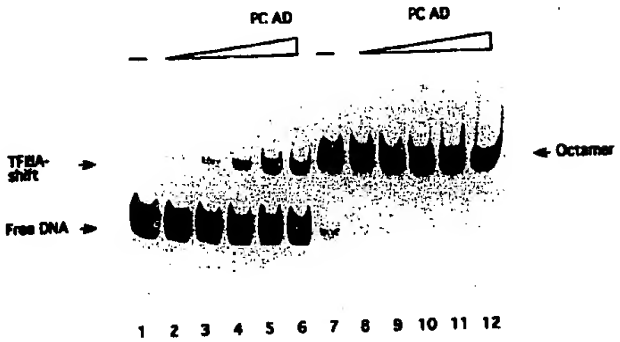

Figure 6. Binding of hTFIIIA to the naked h5S DNA fragment or to the same fragment bearing the reconstituted nucleosomal core, analyzed by electrophoretic mobility shift. 1 ng of radiolabelled h5S DNA or the respective nucleosomal core were preincubated with 5, 10, 20, 30 or 40 µl of PC AD fraction (lanes 2–6 and 8–12, respectively). Lanes 1 and 7 correspond to the h5S DNA and the octamer without PC AD fraction (see Materials and Methods for the detailed binding reaction conditions).

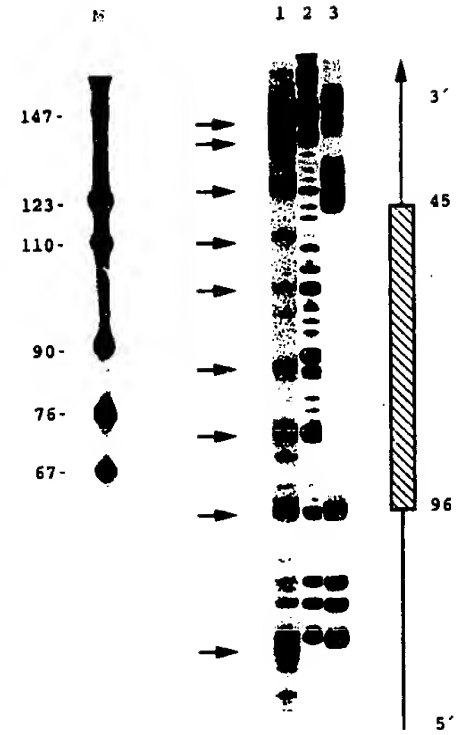

Figure 7. Footprint analyses of the reconstituted nucleosomal h5S DNA shown in Figure 6. The 5S DNA fragment containing a mononucleosome was treated with DNAse I as described in Materials and Methods (lane 1). As a control, the free 5S DNA fragment was incubated without (lane 2) or with hTFIIIA (lane 3) and likewise digested with DNAse I. Lane M depicts a standard marker (pBr 322, *Msp*I digest) to calibrate the gels. The hatched box of the vertical arrow outlines the hTFIIIA footprint on the coding strand of the human 5S gene. The horizontal arrows indicate the DNA fragments generated by DNAse I cleavage of the 5S octamer, depicting the nucleosomal arrangement over the ICR of the 5S promoter.

described above and explains why hTFIIIA is unable to bind to the human 5S promoter under these conditions.

DISCUSSION

Previous experiments demonstrated that several pol II transcription factors can relieve the nucleosomal inhibition of transcription when present in a pre-incubation step (50–53). Similar results were obtained with pol III transcription factors preventing nucleosomal repression of 5S rRNA gene transcription (8,9). However, the factors required for the formation of transcriptionally active 5S rRNA gene chromatin are controversially discussed. Gottesfeld and Bloomer showed for the *X.laevis* oocyte-type 5S gene that TFIIIA alone is able to prevent nucleosomal inhibition when bound to the ICR prior to chromatin assembly (25). In contrast, Tremethick *et al.* (26) found that formation of a stable transcription complex consisting of TFIIIA, TFIIIB and TFIIIC is required to prevent nucleosomal repression of a somatic *X.laevis* 5S gene. Similar results were reported for the yeast 5S gene system (27), also indicating that a complex of TFIIIA, IIIB and IIIC is needed.

In contrast to the extensively studied *Xenopus* and yeast systems, little is known about the role which human TFIIIA plays in connection with the formation of chromatin on the human 5S gene. The gene coding for human TFIIIA has thus far not been cloned because of the low abundance of TFIIIA in human somatic cells. The protein was purified from HeLa cells and characterized with respect to some of its biochemical properties (32,47). From these data and recently published results of Roeder and coworkers (33) it is clear that the human protein differs in molecular weight from the functionally equivalent counterpart in amphibian oocytes. This is in agreement with the findings reported for yeast TFIIIA, which has a distinctly higher mass (50 kDa) and an entirely different amino acid sequence, although the resulting structure, based on nine zinc fingers, is probably very similiar (34,35). In view of the apparent species differences observed in the TFIIIA molecule, we attempted to investigate the participation of the human protein in preventing nucleosomal repression of 5S gene transcription.

Human TFIIIA, purified from HeLa cytoplasmic extracts as described (32), was pre-incubated with the human 5S gene prior to nucleosome assembly by *X.laevis* oocyte extract (S150). The oocyte extract conventionally used in these and previously published experiments is very crude and contains many other proteins besides the histones and assembly factors. To exclude conceivable experimental artefacts due to contaminants, as well as other transcription factors contained in the S150, we additionally focused our attention on an alternative chromatin reconstitution system, which is more defined with respect to its protein composition (43,44). In contrast to the *X.laevis* S150 extract, this reconstitution system contains no transcription factors and hence the anti-repressive effect observed is limited to the proteins contained in the fractions used for pre-incubation. In comparison to the physiological spacing achieved by the S150 extract, this system led to a more dense packaging of nucleosomes after reconstitution. Although based on entirely different techniques, both reconstitution methods yielded similar results, showing that the transcriptional repression associated with formation of nucleosomes could be fully relieved by pre-incubating the template with a fraction highly enriched in hTFIIIA and free of cross-contamination with hTFIIIB and hTFIIIC.

The accessibility of the 5S gene for a restriction enzyme was used as an additional approach to analyse packaging of the ICR

into nucleosomes (54). The decrease in restriction efficiency at a site within the ICR in the presence of increasing amounts of oocyte extract demonstrated that the ICR of the human 5S gene is covered by a nucleosome and is hence inaccessible. These results are in good agreement with those of Gottesfeld and Bloomer (25), who found that *X.laevis* TFIIIA alone mediated the anti-repressive effect.

Another question addressed was the specific binding of hTFIIIA to nucleosomally organized 5S DNA, which is controversially discussed in the literature. TFIIIA from *X.laevis* was previously found to be incapable of binding to a reconstituted histone octamer covering the whole ICR of a somatic *X.laevis* 5S gene (38). In contrast, the formation of a triple complex between TFIIIA and the histone octamer formed on a somatic *X.borealis* 5S gene reported by Rhodes seemed to contradict this result (37).

In order to evaluate the binding of human TFIIIA to a nucleosome, we reconstituted a mononucleosome over a 244 bp fragment of the human 5S gene. In contrast to the free DNA fragment, we observed no detectable binding of hTFIIIA to the DNA complexed in the histone octamer and therefore the results of Rhodes (37) were not reproducible in our assay. The difference between our results and those from Rhodes may, among other reasons, depend on the rotational and translational phasing of the ICR over the nucleosomal core or could be due to species-specific differences in the ICR sequence or biochemical properties of TFIIIA. To shed light on this aspect we investigated the translational phasing of the octamer over the human 5S gene by DNAse I footprinting. In relation to the footprint of hTFIIIA it is evident that the nucleosome covers the entire ICR of the human 5S gene with a 3′ border approximately at position +120, which is very similar to what was described for the nucleosome on the somatic *X.laevis* 5S gene (38). This observation could explain the inability of hTFIIIA to form a ternary complex with the octamer and the human 5S gene, as opposed to the results presented by Rhodes (37) with a somatic *X.borealis* 5S gene, in which case the ICR was only partially covered by the nucleosome, thus retaining the opportunity for TFIIIA to bind to a critical position of the ICR. This particular positioning of the nucleosome in the case of the somatic *X.borealis* 5S gene was confirmed by Hayes *et al.* (55,56). However, these authors have also reported that the histone octamer precluded subsequent binding of TFIIIA to the somatic *X.borealis* 5S gene, whereas the tetramer lacking H2A–H2B dimers allowed its binding (39), and these authors have suggested that the modification and/or integrity of the histones used to reconstitute the nucleosome may explain the contradictory results (57).

In conclusion, we show here for the first time, that human TFIIIA is a strong anti-repressor of nucleosomal inhibition of 5S transcription without the need to form the entire initiation complex as described for the yeast 5S gene (27). Data from EMSA show that hTFIIIA is unable to bind to the ICR once this element is organized in a nucleosome. This is in good agreement with the observation that chromatin assembly completely abolishes transcription of the human 5S gene. Since it is presently impossible to demonstrate that the chromatin assembly methods used in our transcription experiments led to the same translational and rotational positioning of the nucleosome over the ICR as was the case in the nucleosomal template used in the band shift assays, we cannot formally prove that nucleosomal repression of transcription is exclusively due to prevention of the sequence-specific binding of hTFIIIA. It should be emphasized, however, that the complete overlap of the histone octamer and the ICR of the human 5S gene could be a valid explanation for rendering the nucleosomal template transcriptionally active only when hTFIIIA has stably bound. If this is not the case, the nucleosome is formed and consequently TFIIIA cannot gain access to its cognate binding site.

It is likely that mechanisms exist *in vivo* which allow a rearrangement or destabilization of nucleosomes, allowing TFIIIA to bind. Protein complexes which are capable of actively modifying chromatin structure have recently been reported (58,59), although their exact relation to the transcription of nucleosomally organized templates is still unclear. It has also been demonstrated that acetylation of the N-terminal tails of core histones increases the probability of TFIIIA binding to the octamer (57), so that consequences of histone modification for the potential transcription of human 5S chromatin remain to be clarified by future experiments.

ACKNOWLEDGEMENTS

We gratefully acknowledge the expert technical assistance of Frauke Seifart, Ursula Kopiniak and Anne Weber. We also thank Martin Teichmann for preparing the anti-hTFIIIA sera. Furthermore we want to acknowledge Miguel Beato, Marburg, for having made available the *X.laevis* oocyte chromatin assembly method and Frank Schäfer for helpful discussions. Financial support from the Deutsche Forschungsgemeinschaft, the EC (Biotec Programme PL 920090) as well as the Fonds der Chemischen Industrie has made these investigations possible and is gratefully acknowledged.

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AN   2000:335547  CAPLUS
DN   133:1495
TI   Human transcription factor IIIA and cDNA and their use in diagnosis and
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IN Bordon-Pallier, Florence; Rocher, Corinne
PA Hoechst Marion Roussel, Fr.
SO PCT Int. Appl., 49 pp.
CODEN: PIXXD2
DT Patent
LA French
FAN.CNT 1

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
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	FR 2785618	A1	20000512	FR 1998-14146	19981110
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	EP 1137775	A1	20011004	EP 1999-971850	19991109
	R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO				
	JP 2002529084	T2	20020910	JP 2000-581191	19991109
PRAI	FR 1998-14146	A	19981110		
	WO 1999-FR2738	W	19991109		

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AN 2002:125709 BIOSIS
DN PREV200200125709
TI HTFIIA gene.
AU Fujiwara, T.; Takeda, S.; Shimada, Y.; Ozaki, K.; Shin, S.
CS Naruto Japan
ASSIGNEE: OTSUKA PHARMACEUTICAL CO., LTD.
PI US 5808030 Sept. 15, 1998
SO Official Gazette of the United States Patent and Trademark Office Patents, (Sept. 15, 1998) Vol. 1214, No. 3, pp. 3014.
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L2 ANSWER 3 OF 9 CAPLUS COPYRIGHT 2003 ACS
AN 1996:252630 CAPLUS
DN 124:281126
TI Human transcription factor TFIIIA
IN Fujiwara, Tsutomu; Takeda, Satoshi; Shimada, Yoshikazu; Ozaki, Kouichi; Shin, Sadahito
PA Otsuka Pharmaceutical Co., Ltd., Japan
SO Eur. Pat. Appl., 17 pp.
CODEN: EPXXDW
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LA English
FAN.CNT 1

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L2 ANSWER 4 OF 9 MEDLINE DUPLICATE 3
AN 95175352 MEDLINE
DN 95175352 PubMed ID: 7870575
TI Human TFIIIA alone is sufficient to prevent nucleosomal repression of a homologous 5S gene.
AU Stunkel W; Kober I; Kauer M; Taimor G; Seifart K H
CS Institut fur Molekularbiologie und Tumorforschung, Phillips Universitat Marburg, Germany.
SO NUCLEIC ACIDS RESEARCH, (1995 Jan 11) 23 (1) 109-16.
Journal code: 0411011. ISSN: 0305-1048.
CY ENGLAND: United Kingdom
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
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ED Entered STN: 19950407
Last Updated on STN: 19950407
Entered Medline: 19950328

L2 ANSWER 5 OF 9 MEDLINE DUPLICATE 4
AN 94342241 MEDLINE
DN 94342241 PubMed ID: 8063702
TI Purification and characterization of human transcription factor IIIA.
AU Moorefield B; Roeder R G
CS Laboratory of Biochemistry and Molecular Biology, Rockefeller University, New York, New York 10021.
NC 5R35 CA42567 (NCI)
CA09673 (NCI)
SO JOURNAL OF BIOLOGICAL CHEMISTRY, (1994 Aug 19) 269 (33) 20857-65.
Journal code: 2985121R. ISSN: 0021-9258.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
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ED Entered STN: 19941005
Last Updated on STN: 19970203
Entered Medline: 19940921

L2 ANSWER 6 OF 9 MEDLINE DUPLICATE 5
AN 91227135 MEDLINE
DN 91227135 PubMed ID: 1902949
TI Human transcription factor IIIC binds to its cognate promoter sequences in a metal coordinated fashion.
AU Waldschmidt R; Schneider H R; Seifart K H
CS Institut fur Molekularbiologie und Tumorforschung, Marburg/Lahn, FRG.
SO NUCLEIC ACIDS RESEARCH, (1991 Apr 11) 19 (7) 1455-9.
Journal code: 0411011. ISSN: 0305-1048.
CY ENGLAND: United Kingdom
DT Journal; Article; (JOURNAL ARTICLE)
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Last Updated on STN: 19970203
Entered Medline: 19910611

L2 ANSWER 7 OF 9 SCISEARCH COPYRIGHT 2003 THOMSON ISI
AN 91:230991 SCISEARCH

GA The Genuine Article (R) Number: FG762
TI HUMAN TRANSCRIPTION FACTOR-IIIC BINDS TO ITS COGNATE PROMOTER SEQUENCES IN A METAL COORDINATED FASHION
AU WALDSCHMIDT R; SCHNEIDER H R; SEIFART K H (Reprint)
CS INST MOLEK BIOL & TUMORFORSCH, KARL VON FRISCH STR, W-3550 MARBURG, GERMANY
CYA GERMANY
SO NUCLEIC ACIDS RESEARCH, (1991) Vol. 19, No. 7, pp. 1455-1459.
DT Article; Journal
FS LIFE
LA ENGLISH
REC Reference Count: 14
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

L2 ANSWER 8 OF 9 MEDLINE DUPLICATE 6
AN 91071188 MEDLINE
DN 91071188 PubMed ID: 2253613
TI Physical and immunological characterization of human transcription factor IIIA.
AU Waldschmidt R; Jahn D; Teichmann M; Jahn M; Meissner W; Seifart K H
CS Institut fur Molekularbiologie und Tumorforschung, Marburg/Lahnberge, Federal Republic of Germany.
SO EUROPEAN JOURNAL OF BIOCHEMISTRY, (1990 Nov 26) 194 (1) 167-76.
Journal code: 0107600. ISSN: 0014-2956.
CY GERMANY: Germany, Federal Republic of
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199101
ED Entered STN: 19910308
Last Updated on STN: 19910308
Entered Medline: 19910124

L2 ANSWER 9 OF 9 MEDLINE DUPLICATE 7
AN 89109186 MEDLINE
DN 89109186 PubMed ID: 2912980
TI Purification of human transcription factor IIIA and its interaction with a chemically synthesized gene encoding human 5 S rRNA.
AU Seifart K H; Wang L; Waldschmidt R; Jahn D; Wingender E
CS Institut fur Molekularbiologie and Tumorforschung, Marburg/Lahnberge, Federal Republic of Germany.
SO JOURNAL OF BIOLOGICAL CHEMISTRY, (1989 Jan 25) 264 (3) 1702-9.
Journal code: 2985121R. ISSN: 0021-9258.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 198903
ED Entered STN: 19900308
Last Updated on STN: 19970203
Entered Medline: 19890303

=> d l1 1-31

L1 ANSWER 1 OF 31 MEDLINE
AN 95175352 MEDLINE
DN 95175352 PubMed ID: 7870575
TI Human TFIIIA alone is sufficient to prevent nucleosomal repression of a homologous 5S gene.
AU Stunkel W; Kober I; Kauer M; Taimor G; Seifart K H
CS Institut fur Molekularbiologie und Tumorforschung, Phillips Universitat Marburg, Germany.
SO NUCLEIC ACIDS RESEARCH, (1995 Jan 11) 23 (1) 109-16.
Journal code: 0411011. ISSN: 0305-1048.
CY ENGLAND: United Kingdom
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199503
ED Entered STN: 19950407
Last Updated on STN: 19950407
Entered Medline: 19950328

L1 ANSWER 2 OF 31 MEDLINE
AN 94342241 MEDLINE
DN 94342241 PubMed ID: 8063702
TI Purification and characterization of human transcription factor IIIA.
AU Moorefield B; Roeder R G
CS Laboratory of Biochemistry and Molecular Biology, Rockefeller University, New York, New York 10021.
NC 5R35 CA42567 (NCI)
CA09673 (NCI)
SO JOURNAL OF BIOLOGICAL CHEMISTRY, (1994 Aug 19) 269 (33) 20857-65.
Journal code: 2985121R. ISSN: 0021-9258.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199409
ED Entered STN: 19941005
Last Updated on STN: 19970203
Entered Medline: 19940921

L1 ANSWER 3 OF 31 MEDLINE
AN 91227135 MEDLINE
DN 91227135 PubMed ID: 1902949
TI Human transcription factor IIIC binds to its cognate promoter sequences in a metal coordinated fashion.
AU Waldschmidt R; Schneider H R; Seifart K H
CS Institut fur Molekularbiologie und Tumorforschung, Marburg/Lahn, FRG.
SO NUCLEIC ACIDS RESEARCH, (1991 Apr 11) 19 (7) 1455-9.
Journal code: 0411011. ISSN: 0305-1048.
CY ENGLAND: United Kingdom
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199106
ED Entered STN: 19910630
Last Updated on STN: 19970203
Entered Medline: 19910611

L1 ANSWER 4 OF 31 MEDLINE
AN 91071188 MEDLINE
DN 91071188 PubMed ID: 2253613
TI Physical and immunological characterization of human transcription factor IIIA.
AU Waldschmidt R; Jahn D; Teichmann M; Jahn M; Meissner W; Seifart K H
CS Institut fur Molekularbiologie und Tumorforschung, Marburg/Lahnberge, Federal Republic of Germany.
SO EUROPEAN JOURNAL OF BIOCHEMISTRY, (1990 Nov 26) 194 (1) 167-76.
Journal code: 0107600. ISSN: 0014-2956.
CY GERMANY: Germany, Federal Republic of
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199101
ED Entered STN: 19910308

Last Updated on STN: 19910308
Entered Medline: 19910124

L1 ANSWER 5 OF 31 MEDLINE
AN 89109186 MEDLINE
DN 89109186 PubMed ID: 2912980
TI Purification of human transcription factor IIIA and its interaction with a chemically synthesized gene encoding human 5 S rRNA.
AU Seifart K H; Wang L; Waldschmidt R; Jahn D; Wingender E
CS Institut fur Molekularbiologie and Tumorforschung, Marburg/Lahnberge, Federal Republic of Germany.
SO JOURNAL OF BIOLOGICAL CHEMISTRY, (1989 Jan 25) 264 (3) 1702-9.
Journal code: 2985121R. ISSN: 0021-9258.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 198903
ED Entered STN: 19900308
Last Updated on STN: 19970203
Entered Medline: 19890303

L1 ANSWER 6 OF 31 CANCERLIT
AN 94342241 CANCERLIT
DN 94342241 PubMed ID: 8063702
TI Purification and characterization of human transcription factor IIIA.
AU Moorefield B; Roeder R G
CS Laboratory of Biochemistry and Molecular Biology, Rockefeller University, New York, New York 10021.
NC 5R35 CA42567 (NCI)
CA09673 (NCI)
SO JOURNAL OF BIOLOGICAL CHEMISTRY, (1994 Aug 19) 269 (33) 20857-65.
Journal code: 2985121R. ISSN: 0021-9258.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS MEDLINE; Priority Journals
OS MEDLINE 94342241
EM 199409
ED Entered STN: 19990618
Last Updated on STN: 19990618

L1 ANSWER 7 OF 31 CANCERLIT
AN 91227135 CANCERLIT
DN 91227135 PubMed ID: 1902949
TI Human transcription factor IIIC binds to its cognate promoter sequences in a metal coordinated fashion.
AU Waldschmidt R; Schneider H R; Seifart K H
CS Institut fur Molekularbiologie und Tumorforschung, Marburg/Lahn, FRG.
SO NUCLEIC ACIDS RESEARCH, (1991 Apr 11) 19 (7) 1455-9.
Journal code: 0411011. ISSN: 0305-1048.
CY ENGLAND: United Kingdom
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS MEDLINE; Priority Journals
OS MEDLINE 91227135
EM 199106
ED Entered STN: 19941107
Last Updated on STN: 19970509

L1 ANSWER 8 OF 31 CANCERLIT
AN 89109186 CANCERLIT
DN 89109186 PubMed ID: 2912980
TI Purification of human transcription factor IIIA and its interaction with a chemically synthesized gene encoding human 5 S rRNA.
AU Seifart K H; Wang L; Waldschmidt R; Jahn D; Wingender E
CS Institut fur Molekularbiologie and Tumorforschung, Marburg/Lahnberge, Federal Republic of Germany.
SO JOURNAL OF BIOLOGICAL CHEMISTRY, (1989 Jan 25) 264 (3) 1702-9.
Journal code: 2985121R. ISSN: 0021-9258.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS MEDLINE; Priority Journals
OS MEDLINE 89109186
EM 198903
ED Entered STN: 19990618
Last Updated on STN: 19990618

L1 ANSWER 9 OF 31 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
AN 2002:125709 BIOSIS
DN PREV200200125709
TI HTFIIA gene.
AU Fujiwara, T.; Takeda, S.; Shimada, Y.; Ozaki, K.; Shin, S.
CS Naruto Japan
ASSIGNEE: OTSUKA PHARMACEUTICAL CO., LTD.
PI US 5808030 Sept. 15, 1998
SO Official Gazette of the United States Patent and Trademark Office Patents, (Sept. 15, 1998) Vol. 1214, No. 3, pp. 3014.
ISSN: 0098-1133.
DT Patent
LA English

L1 ANSWER 10 OF 31 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
AN 1995:171773 BIOSIS
DN PREV199598186073
TI Human TFIIIA alone is sufficient to prevent nucleosomal repression of a homologous 5S gene.
AU Stuenkel, Walter; Kober, Ingo; Kauer, Manfred; Taimor, Gerhild; Seifart, Klaus H. (1)
CS (1) Inst. Molekularbiolgie und Tumorforschung, Phillips Univ. Marburg, Lahnstrasse 3, D-35037 Marburg Germany
SO Nucleic Acids Research, (1995) Vol. 23, No. 1, pp. 109-116.
ISSN: 0305-1048.
DT Article
LA English

L1 ANSWER 11 OF 31 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
AN 1994:435986 BIOSIS
DN PREV199497448986
TI Purification and characterization of human transcription factor IIIA.
AU Moorefield, Beth; Roeder, Robert G. (1)
CS (1) Lab. Biochem. Mol. Biol., Rockefeller Univ., New York, NY 10021 USA
SO Journal of Biological Chemistry, (1994) Vol. 269, No. 33, pp. 20857-20865.
ISSN: 0021-9258.
DT Article
LA English

L1 ANSWER 12 OF 31 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
AN 1991:271800 BIOSIS
DN BA92:4415
TI HUMAN TRANSCRIPTION FACTOR IIIC BINDS TO ITS COGNATE PROMOTER SEQUENCES IN A METAL COORDINATED FASHION.
AU WALDSCHMIDT R; SCHNEIDER H R; SEIFART K H
CS INST. MOLEKULARBIOL. UND TUMORFORSCHUNG, KARL-VON-FRISCH STRASSE, D 3550 MARBURG/LAHN, FRG.
SO NUCLEIC ACIDS RES, (1991) 19 (7), 1455-1460.
CODEN: NARHAD. ISSN: 0305-1048.

FS BA; OLD
LA English

L1 ANSWER 13 OF 31 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
AN 1991:87872 BIOSIS
DN BA91:46762
TI PHYSICAL AND IMMUNOLOGICAL CHARACTERIZATION OF HUMAN TRANSCRIPTION FACTOR IIIA.
AU WALDSCHMIDT R; JAHN D; TEICHMANN M; JAHN M; MEISSNER W; SEIFART K H
CS INSTITUT MOLEKULARBIOLOGIE TUMORFORSCHUNG, KARL VON FRISCH-STRASSE, W-3550 MARBURG/LAHNBERGE, W. GER.
SO EUR J BIOCHEM, (1990) 194 (1), 167-176.
CODEN: EJBCAI. ISSN: 0014-2956.
FS BA; OLD
LA English

L1 ANSWER 14 OF 31 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
AN 1989:135939 BIOSIS
DN BA87:70592
TI PURIFICATION OF HUMAN TRANSCRIPTION FACTOR IIA AND ITS INTERACTION WITH A CHEMICALLY SYNTHESIZED GENE ENCODING HUMAN 5S RIBOSOMAL RNA.
AU SEIFART K H; WANG L; WALDSCHMIDT R; JAHN D; WINGENDER E
CS INST. FUER MOLEKULARBIOL. AND TUMORFORSCHUNG, KARL-VON-FRISCH-STRASSE, D-3550 MARBURG/LAHNBERGE, F.R.G.
SO J BIOL CHEM, (1989) 264 (3), 1702-1709.
CODEN: JBCHA3. ISSN: 0021-9258.
FS BA; OLD
LA English

L1 ANSWER 15 OF 31 EMBASE COPYRIGHT 2003 ELSEVIER SCI. B.V.
AN 95060457 EMBASE
DN 1995060457
TI Human TFIIIA alone is sufficient to prevent nucleosomal repression of a homologous 5S gene.
AU Stunkel W.; Kober I.; Kauer M.; Taimor G.; Seifart K.H.
CS Inst Molekularbiol Tumorforschung, Phillips Universitat Marburg, Lahnstrasse 3,D-35037 Marburg, Germany
SO Nucleic Acids Research, (1995) 23/1 (109-116).
ISSN: 0305-1048 CODEN: NARHAD
CY United Kingdom
DT Journal; Article
FS 022 Human Genetics
LA English
SL English

L1 ANSWER 16 OF 31 EMBASE COPYRIGHT 2003 ELSEVIER SCI. B.V.
AN 94264117 EMBASE
DN 1994264117
TI Purification and characterization of human transcription factor IIIA.
AU Moorefield B.; Roeder R.G.
CS Biochemistry/Molecular Biology Lab., Rockefeller University,New York, NY 10021, United States
SO Journal of Biological Chemistry, (1994) 269/33 (20857-20865).
ISSN: 0021-9258 CODEN: JBCHA3
CY United States
DT Journal; Article
FS 029 Clinical Biochemistry
LA English
SL English

L1 ANSWER 17 OF 31 EMBASE COPYRIGHT 2003 ELSEVIER SCI. B.V.
AN 91138089 EMBASE
DN 1991138089
TI Human transcription factor IIIC binds to its cognate promoter sequences in a metal coordinated fashion.
AU Waldschmidt R.; Schneider H.R.; Seifart K.H.
CS Inst. fur Molekularbiologie, und Tumorforschung, Karl-von-Frisch Strasse,D 3550 Marburg/Lahn, Germany
SO Nucleic Acids Research, (1991) 19/7 (1455-1459).
ISSN: 0305-1048 CODEN: NARHAD
CY United Kingdom
DT Journal; Article
FS 029 Clinical Biochemistry
LA English
SL English

L1 ANSWER 18 OF 31 EMBASE COPYRIGHT 2003 ELSEVIER SCI. B.V.
AN 91002299 EMBASE
DN 1991002299
TI Physical and immunological characterization of human transcription factor IIIA.
AU Waldschmidt R.; Jahn D.; Teichmann M.; Jahn M.; Meissner W.; Seifart K.H.
CS Inst. fur Molekularbiologie, und Tumorforschung, Karl von Frisch-Strasse,W-3550 Marburg/Lahnberge, Germany
SO European Journal of Biochemistry, (1990) 194/1 (167-176).
ISSN: 0014-2956 CODEN: EJBCAI
CY Germany
DT Journal; Article
FS 029 Clinical Biochemistry
LA English
SL English

L1 ANSWER 19 OF 31 EMBASE COPYRIGHT 2003 ELSEVIER SCI. B.V.
AN 89051014 EMBASE
DN 1989051014
TI Purification of human transcription factor IIIA and its interaction with a chemically synthesized gene encoding human 5 S rRNA.
AU Seifart K.H.; Wang L.; Waldschmidt R.; Jahn D.; Wingender E.
CS Institut fur Molekularbiologie and Tumorforsuchung, D-3550 Marburg/Lahnberge, Germany
SO Journal of Biological Chemistry, (1989) 264/3 (1702-1709).
ISSN: 0021-9258 CODEN: JBCHA3
CY United States
DT Journal
FS 029 Clinical Biochemistry
LA English
SL English

L1 ANSWER 20 OF 31 CAPLUS COPYRIGHT 2003 ACS
AN 2000:335547 CAPLUS
DN 133:1495
TI Human transcription factor IIIA and cDNA and their use in diagnosis and therapy
IN Bordon-Pallier, Florence; Rocher, Corinne
PA Hoechst Marion Roussel, Fr.
SO PCT Int. Appl., 49 pp.
CODEN: PIXXD2
DT Patent
LA French
FAN.CNT 1

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	WO 2000028024	A1	20000518	WO 1999-FR2738	19991109

W: AE, AL, AU, BA, BB, BG, BR, CA, CN, CR, CU, CZ, DM, EE, GD, GE, HR, HU, ID, IL, IN, IS, JP, KP, KR, LC, LK, LR, LT, LV, MA, MG, MK, MN, MX, NO, NZ, PL, RO, SG, SI, SK, SL, TR, TT, UA, US, UZ, VN, YU, ZA, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM
RW: GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE,

DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG

	FR 2785618	A1	20000512	FR 1998-14146	19981110
	FR 2785618	B1	20021213		
	EP 1137775	A1	20011004	EP 1999-971850	19991109
	R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO				
	JP 2002529084	T2	20020910	JP 2000-581191	19991109
PRAI	FR 1998-14146	A	19981110		
	WO 1999-FR2738	W	19991109		

RE.CNT 7 THERE ARE 7 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L1 ANSWER 21 OF 31 CAPLUS COPYRIGHT 2003 ACS
AN 1996:252630 CAPLUS
DN 124:281126
TI Human transcription factor TFIIIA
IN Fujiwara, Tsutomu; Takeda, Satoshi; Shimada, Yoshikazu; Ozaki, Kouichi; Shin, Sadahito
PA Otsuka Pharmaceutical Co., Ltd., Japan
SO Eur. Pat. Appl., 17 pp.
CODEN: EPXXDW
DT Patent
LA English
FAN.CNT 1

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	EP 704526	A1	19960403	EP 1995-113908	19950905
	EP 704526	B1	20001220		
	R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LI, LU, MC, NL, PT, SE				
	JP 08070870	A2	19960319	JP 1994-211022	19940905
	JP 2946019	B2	19990906		
	CA 2157531	AA	19960306	CA 1995-2157531	19950905
	CN 1134460	A	19961030	CN 1995-117131	19950905
	US 5808030	A	19980915	US 1995-523376	19950905
	MX 9503806	A	20000630	MX 1995-3806	19950905
	AT 198218	E	20010115	AT 1995-113908	19950905
PRAI	JP 1994-211022	A	19940905		

L1 ANSWER 22 OF 31 CAPLUS COPYRIGHT 2003 ACS
AN 1995:439437 CAPLUS
DN 122:257820
TI Human TFIIIA alone is sufficient to prevent nucleosomal repression of a homologous 5S gene
AU Stuenkel, Walter; Kober, Ingo; Kauer, Manfred; Taimor, Gerhild; Seifart, Klaus H.
CS Inst. Mol. Tumorforschung, Phillips Univ. Marburg, Marburg, D-35037, Germany
SO Nucleic Acids Research (1995), 23(1), 109-16
CODEN: NARHAD; ISSN: 0305-1048
PB Oxford University Press
DT Journal
LA English

L1 ANSWER 23 OF 31 CAPLUS COPYRIGHT 2003 ACS
AN 1994:552762 CAPLUS
DN 121:152762
TI Purification and characterization of human transcription factor IIIA
AU Moorefield, Beth; Roeder, Robert G.
CS Lab. Biochem. Mol. Biol., Rockefeller Univ., New York, NY, 10021, USA
SO Journal of Biological Chemistry (1994), 269(33), 20857-65
CODEN: JBCHA3; ISSN: 0021-9258
DT Journal
LA English

L1 ANSWER 24 OF 31 CAPLUS COPYRIGHT 2003 ACS
AN 1991:241794 CAPLUS
DN 114:241794
TI Human transcription factor IIIC binds to its cognate promoter sequences in a metal coordinated fashion
AU Waldschmidt, Rainer; Schneider, Harald R.; Seifart, Klaus H.
CS Inst. Molekularbiol. Tumorforsch., Marburg/Lahn, D 3550, Germany
SO Nucleic Acids Research (1991), 19(7), 1455-9
CODEN: NARHAD; ISSN: 0305-1048
DT Journal
LA English

L1 ANSWER 25 OF 31 CAPLUS COPYRIGHT 2003 ACS
AN 1991:38065 CAPLUS
DN 114:38065
TI Physical and immunological characterization of human transcription factor IIIA
AU Waldschmidt, Rainer; Jahn, Dieter; Teichmann, Martin; Jahn, Martina; Meissner, Wolfgang; Seifart, Klaus H.
CS Inst. Molekularbiol. Tumorforsch., Marburg/Lahnberge, Germany
SO European Journal of Biochemistry (1990), 194(1), 167-76
CODEN: EJBCAI; ISSN: 0014-2956
DT Journal
LA English

L1 ANSWER 26 OF 31 CAPLUS COPYRIGHT 2003 ACS
AN 1989:129679 CAPLUS
DN 110:129679
TI Purification of human transcription factor IIIA and its interaction with a chemically synthesized gene encoding human 5 S rRNA
AU Seifart, Klaus H.; Wang, Lingru; Waldschmidt, Rainer; Jahn, Dieter; Wingender, Edgar
CS Inst. Molekularbiol. Tumorforsch., Marburg, D-3550, Fed. Rep. Ger.
SO Journal of Biological Chemistry (1989), 264(3), 1702-9
CODEN: JBCHA3; ISSN: 0021-9258
DT Journal
LA English

L1 ANSWER 27 OF 31 USPATFULL
AN 1998:112101 USPATFULL
TI **hTFIIIA** gene
IN Fujiwara, Tsutomu, Naruto, Japan
Takeda, Satoshi, Tokushima, Japan
Shimada, Yoshikazu, Tokushima, Japan
Ozaki, Kouichi, Tokyo, Japan
Shin, deceased, Sadahito, late of Tokushima-ken, Japan by Sadae Kim, legal representative
PA Otsuka Pharmaceutical Co., Ltd., Tokyo, Japan (non-U.S. corporation)
PI US 5808030 19980915
AI US 1995-523376 19950905 (8)
PRAI JP 1994-211022 19940905
DT Utility
FS Granted
LN.CNT 623
INCL INCLM: 536/023.500
NCL NCLM: 536/023.500
IC [6]
ICM: C07H021-04
EXF 536/23.5
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L1 ANSWER 28 OF 31 PCTFULL COPYRIGHT 2003 Univentio
AN 2000028024 PCTFULL ED 20020515

TIEN htfIIIA HUMAN GENE AND CODED hTFIIIA PROTEIN
TIFR GENE HUMAIN htfIIIA ET LA PROTEINE CODEE hTFIIIA
IN BORDON-PALLIER, Florence;
ROCHER, Corinne
PA HOECHST MARION ROUSSEL;
BORDON-PALLIER, Florence;
ROCHER, Corinne
LA French
DT Patent
PI WO 2000028024 A1 20000518
DS W: AE AL AU BA BB BG BR CA CN CR CU CZ DM EE GD GE HR HU ID IL
IN IS JP KP KR LC LK LR LT LV MA MG MK MN MX NO NZ PL RO SG
SI SK SL TR TT UA US UZ VN YU ZA GH GM KE LS MW SD SL SZ TZ
UG ZW AM AZ BY KG KZ MD RU TJ TM AT BE CH CY DE DK ES FI FR
GB GR IE IT LU MC NL PT SE BF BJ CF CG CI CM GA GN GW ML MR
NE SN TD TG
AI WO 1999-FR2738 A 19991109
PRAI FR 1998-98/14146 19981110
ICM C12N015-12
ICS C07K014-47; C12Q001-68; A61K038-17; G01N033-50; A61K048-00

L1 ANSWER 29 OF 31 SCISEARCH COPYRIGHT 2003 THOMSON ISI
AN 95:151918 SCISEARCH
GA The Genuine Article (R) Number: QH281
TI HUMAN TFIIIA ALONE IS SUFFICIENT TO PREVENT NUCLEOSOMAL REPRESSION OF A HOMOLOGOUS 5S GENE
AU STUNKEL W; KOBER I; KAUER M; TAIMOR G; SEIFART K H (Reprint)
CS UNIV MARBURG, INST MOLEK BIOL & TUMORFORSCH, LAHNSTR 3, D-35037 MARBURG, GERMANY (Reprint); UNIV MARBURG, INST MOLEK BIOL & TUMORFORSCH, D-35037 MARBURG, GERMANY
CYA GERMANY
SO NUCLEIC ACIDS RESEARCH, (11 JAN 1995) Vol. 23, No. 1, pp. 109-116.
ISSN: 0305-1048.
DT Article; Journal
FS LIFE
LA ENGLISH
REC Reference Count: 58
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

L1 ANSWER 30 OF 31 SCISEARCH COPYRIGHT 2003 THOMSON ISI
AN 94:500220 SCISEARCH
GA The Genuine Article (R) Number: PC403
TI PURIFICATION AND CHARACTERIZATION OF HUMAN TRANSCRIPTION FACTOR IIIA
AU MOOREFIELD B; ROEDER R G (Reprint)
CS ROCKEFELLER UNIV, BIOCHEM & MOLEC BIOL LAB, NEW YORK, NY, 10021 (Reprint); ROCKEFELLER UNIV, BIOCHEM & MOLEC BIOL LAB, NEW YORK, NY, 10021
CYA USA
SO JOURNAL OF BIOLOGICAL CHEMISTRY, (19 AUG 1994) Vol. 269, No. 33, pp. 20857-20865.
ISSN: 0021-9258.
DT Article; Journal
FS LIFE
LA ENGLISH
REC Reference Count: 57
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

L1 ANSWER 31 OF 31 SCISEARCH COPYRIGHT 2003 THOMSON ISI
AN 91:230991 SCISEARCH
GA The Genuine Article (R) Number: FG762
TI HUMAN TRANSCRIPTION FACTOR-IIIC BINDS TO ITS COGNATE PROMOTER SEQUENCES IN A METAL COORDINATED FASHION
AU WALDSCHMIDT R; SCHNEIDER H R; SEIFART K H (Reprint)
CS INST MOLEK BIOL & TUMORFORSCH, KARL VON FRISCH STR, W-3550 MARBURG, GERMANY
CYA GERMANY
SO NUCLEIC ACIDS RESEARCH, (1991) Vol. 19, No. 7, pp. 1455-1459.
DT Article; Journal
FS LIFE
LA ENGLISH
REC Reference Count: 14
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

WEST Search History

DATE: Tuesday, May 20, 2003

Set Name side by side	Query	Hit Count	Set Name result set
DB=USPT,PGPB,JPAB,EPAB,DWPI; PLUR=YES; OP=OR			
L2	BORDON adj PALLIER	6	L2
L1	htfIIIA	6	L1

END OF SEARCH HISTORY

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              0  HTF3A
             28  HTFIIIA
      S1     28  HTF3A OR HTFIIIA
?rd
...completed examining records
      S2      8  RD (unique items)
```

2/3/1 (Item 1 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

09731155 BIOSIS NO.: 199598186073
Human TFIIIA alone is sufficient to prevent nucleosomal repression of a homologous 5S gene.
AUTHOR: Stuenkel Walter; Kober Ingo; Kauer Manfred; Taimor Gerhild; Seifart Klaus H(a)
AUTHOR ADDRESS: (a)Inst. Molekularbiolgie und Tumorforschung, Phillips Univ. Marburg, Lahnstrasse 3, D-35037 Marbur**Germany
JOURNAL: Nucleic Acids Research 23 (1):p109-116 1995
ISSN: 0305-1048
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

2/3/2 (Item 2 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

09440616 BIOSIS NO.: 199497448986
Purification and characterization of human transcription factor IIIA.
AUTHOR: Moorefield Beth; Roeder Robert G(a)
AUTHOR ADDRESS: (a)Lab. Biochem. Mol. Biol., Rockefeller Univ., New York, NY 10021**USA
JOURNAL: Journal of Biological Chemistry 269 (33):p20857-20865 1994
ISSN: 0021-9258
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

2/3/4 (Item 4 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

07450543 BIOSIS NO.: 000091046762
PHYSICAL AND IMMUNOLOGICAL CHARACTERIZATION OF HUMAN TRANSCRIPTION FACTOR IIIA
AUTHOR: WALDSCHMIDT R; JAHN D; TEICHMANN M; JAHN M; MEISSNER W; SEIFART K H
AUTHOR ADDRESS: INSTITUT MOLEKULARBIOLOGIE TUMORFORSCHUNG, KARL VON FRISCH-STRASSE, W-3550 MARBURG/LAHNBERGE, W. GER.
JOURNAL: EUR J BIOCHEM 194 (1). 1990. 167-176. 1990
FULL JOURNAL NAME: European Journal of Biochemistry
CODEN: EJBCA
RECORD TYPE: Abstract
LANGUAGE: ENGLISH

2/3/5 (Item 5 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

06628430 BIOSIS NO.: 000087070592
PURIFICATION OF HUMAN TRANSCRIPTION FACTOR IIA AND ITS INTERACTION WITH A CHEMICALLY SYNTHESIZED GENE ENCODING HUMAN 5S RIBOSOMAL RNA
AUTHOR: SEIFART K H; WANG L; WALDSCHMIDT R; JAHN D; WINGENDER E
AUTHOR ADDRESS: INST. FUER MOLEKULARBIOL. AND TUMORFORSCHUNG, KARL-VON-FRISCH-STRASSE, D-3550 MARBURG/LAHNBERGE, F.R.G.
JOURNAL: J BIOL CHEM 264 (3). 1989. 1702-1709. 1989
FULL JOURNAL NAME: Journal of Biological Chemistry
CODEN: JBCHA

RECORD TYPE: Abstract
LANGUAGE: ENGLISH

2/3/6 (Item 1 from file: 73)
DIALOG(R)File 73:EMBASE

03882058 EMBASE No: 1989051014
Purification of human transcription factor IIIA and its interaction with a chemically synthesized gene encoding human 5 S rRNA
Seifart K.H.; Wang L.; Waldschmidt R.; Jahn D.; Wingender E.
Institut fur Molekularbiologie and Tumorforsuchung, D-3550 Marburg/Lahnberge Germany
Journal of Biological Chemistry (J. BIOL. CHEM.) (United States) 1989, 264/3 (1702-1709)
CODEN: JBCHA ISSN: 0021-9258
DOCUMENT TYPE: Journal
LANGUAGE: ENGLISH SUMMARY LANGUAGE: ENGLISH

2/3/7 (Item 1 from file: 315)
DIALOG(R)File 315:ChemEng & Biotec Abs

389997 CEABA Accession No.: 27-07-014421 DOCUMENT TYPE: Patent
Title: hTFIIIA **gene.**
AUTHOR: Fujiwara, Tsutomu ; Takeda, Satoshi ; Shimada, Yoshikazu ; Ozaki, Kouichi ; Shin, Sadahito
CORPORATE SOURCE: Otsuka Pharm. Co. Ltd. Tokyo 101 Japan
CODEN: EPXXDW
PATENT NUMBER: EP 704526
PUBLICATION DATE: 3 Apr 1996 (960403) LANGUAGE: English
PRIORITY PATENT APPLICATION(S) & DATE(S): JP 21102294 (940905)

2/3/3 (Item 3 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

07634471 BIOSIS NO.: 000092004415
HUMAN TRANSCRIPTION FACTOR IIIC BINDS TO ITS COGNATE PROMOTER SEQUENCES IN A METAL COORDINATED FASHION
AUTHOR: WALDSCHMIDT R; SCHNEIDER H R; SEIFART K H
AUTHOR ADDRESS: INST. MOLEKULARBIOL. UND TUMORFORSCHUNG, KARL-VON-FRISCH STRASSE, D 3550 MARBURG/LAHN, FRG.
JOURNAL: NUCLEIC ACIDS RES 19 (7). 1991. 1455-1460. 1991
FULL JOURNAL NAME: Nucleic Acids Research
CODEN: NARHA
RECORD TYPE: Abstract
LANGUAGE: ENGLISH